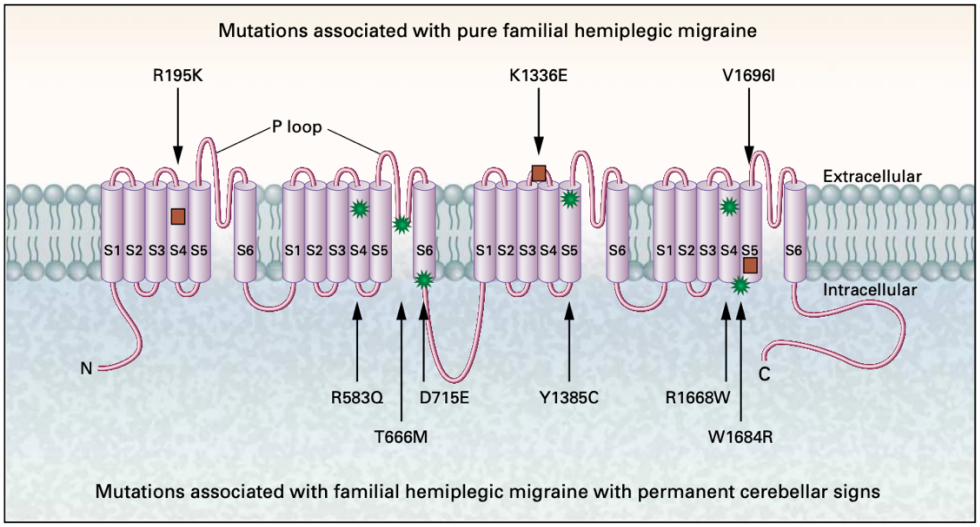
Analysis of R195K Variant

Background

The CACNA1A gene encodes the voltage-dependent P/Q-type (CaV2.1) calcium channel subunit alpha-1A. This subunit is a part of voltage-sensitive calcium channels (VSCC) which control the flow of calcium ions into a cells and allow for many functions that are dependent on calcium such as muscular contractions, release of neurotransmitters, expression of genes, motility, division, and cell death. The alpha-1A subunit create P and Q calcium currents which are considered "high-voltage activated"[1]. The disease variant assigned to me is the R195K variant. This variant is caused by nucleotide mutation from AGG->AAG, which causes a change of residue at position 195 from an Arginine to a Lysine. These two residues have similar properties of size and pH (large and basic), but also introduce a slight change to hydrophobicity, with Lysine being slightly more hydrophilic than Arginine. Despite these similarities, the R195K variant is associated with familial hemiplegic migraines (FHM1)[2]. Variant R195K is located within the transmembrane S4 helical domain of repeat 1 within the CACNA1A protein as shown in the figure below[3].



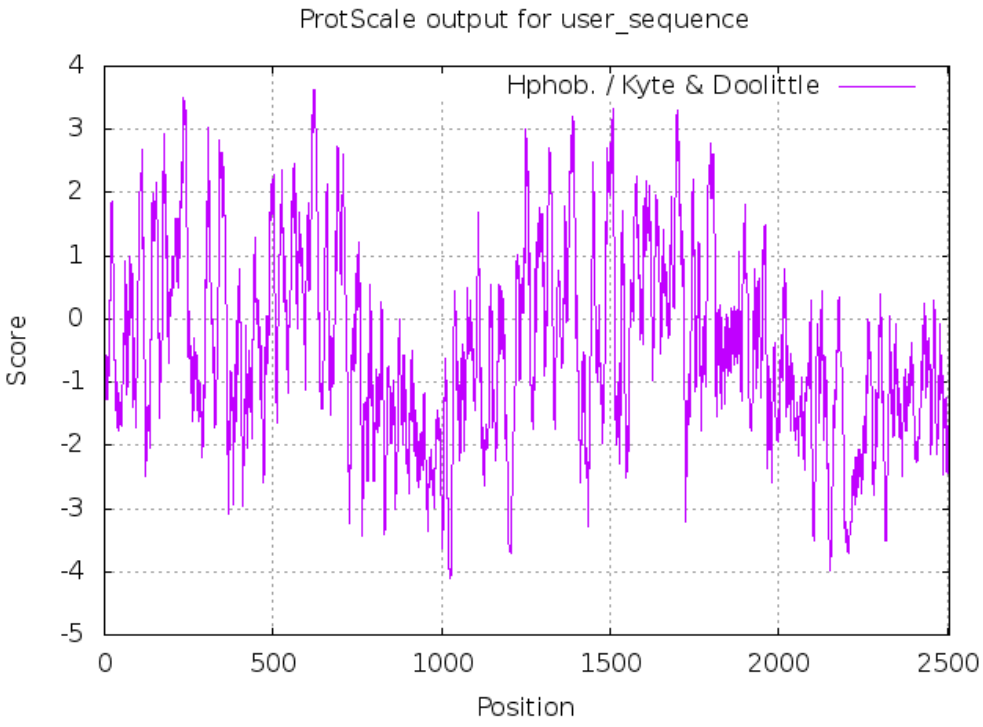
In the paper by Ducros, et al., which discovered the R195K variant of the CACNA1A protein, the authors had found 5 patients containing this SNP and all 5 of these patients suffered from hemiplegic migraines[3]. The cause of hemiplegic migraines stems from mutations found in the CACNA1A, ATP1A2, SCN1A, and PRRT2 genes. In the case of CACNA1A, the protein is important in the shuttling of calcium ions across the cellular membrane and as such, a mutation in CACNA1A can cause dysregulated calcium influx or outflux which causes a disruption in neurotransmitter release and uptake and leads to hemiplegic migraines[4]. The particular area of the CACNA1A protein that the R195K variant affects is that of the Cav2.1 channel, a subset of Cav channels that are responsible for the transportation of calcium ions across the cell membrane. All Cav channel subtypes can trigger CDI, or calcium-dependent inactivation. This is a feedback loop in which the Cav channels can sense the amounts of calcium present intracellularly and either open or close to allow more in or to stop the influx of calcium. In the case of Cav2 channels, they require the opening of multiple channels for an increase of global calcium levels in the intracellular space to then activate low-affinity binding CaM to trigger their CDI. Additionally, Cav2.1 channels have a second calcium regulation pathway called calcium-dependent facilitation (CDF). This pathway uses high-affinity CaM sites in which, unlike CDI, only requires a local increase in calcium to promote CDF. Both CDI and CDF rely on CaM to

regulate calcium influx. Cav2 channels are highly relied upon in synaptic communication and transfer in the central nervous system, thus making them extremely important in normal brain function. It has been observed that mice with Cav2.1 deletions display sever ataxia and absence seizures and die around 4 weeks post-natally. It has also been observed that in humans, a loss of function mutation in the Cav2.1 gene generally leads to severe ataxia and absence seizures just like their mouse counterparts, but gain of function mutations generally leads to spreading cortical depression and migraines[5].

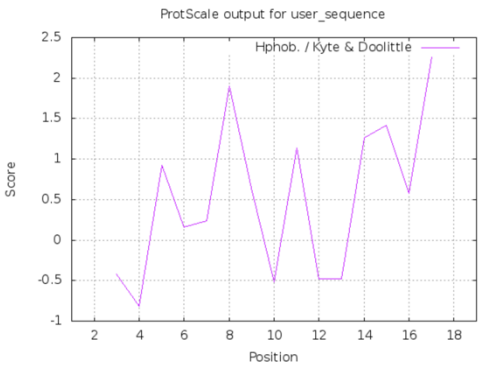
As discussed previously, the root cause of hemiplegic migraines is the dysregulation of calcium ion influx which then leads to a dysregulation of neurotransmitter release or uptake. It is important to take note of the location of the R195K variant. It is located in the S4 helical domain, which is responsible for the control over the voltage-dependent activation of the Cav2.1 channel. According to GHR, the many mutations in the CACNA1A gene that are responsible for familial hemiplegic migraines all change the function and structure of the Cav2.1 channel. These mutations all cause the Cav2.1 channels to open easier than normal and allow more calcium ions into the intracellular space which then increases the release of intracellular neurotransmitters[6].

Protein/Variant Analysis

To begin with, I analyzed basic information about the CACNA1A subunit S4 of repeat I and the R195K variant of the same subunit using ExPASy ProtParam. Through this analysis, I found that the MW between the normal subunit and the R195K variant only changes by about 1.3%, going from 2160.72 (normal) to 2132.71 (variant). Additionally, from ProtParam I was able to determine the theoretical pI of the subunit and found that it went from 12.48 (normal) to 12.31 (variant), a change of only 1.36%. These results do not indicate too much, but also make quite a bit of sense as this is only a point mutation between two relatively similar amino acids[7]. Next, I examined the hydropathicity plot calculated for the CACNA1A gene as a whole and then the normal and variant subunit using ExPASy Protscale. The entire CACNA1A gene hydropathicity plot was calculated using the Kyte & Doolittle index with a sliding window of 9:

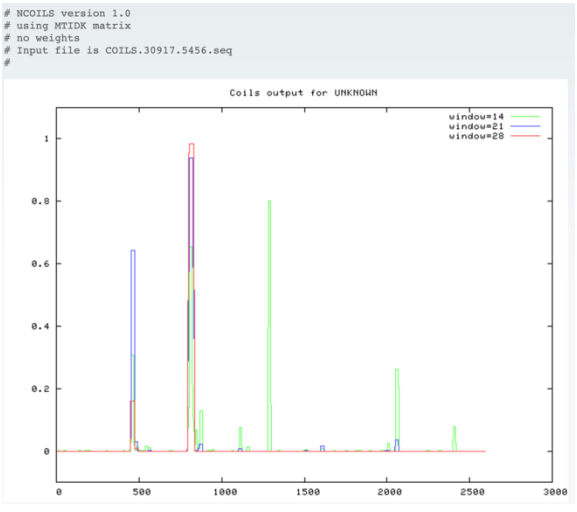


The take away from this plot is that there are significant hydrophobic regions from ~750-1100 and ~1750-2500. Hydrophilic areas: ~150-250, 500-650, 1250-1400, 1550-1750[9]. Hydrophobic regions are generally nonpolar and unable to interact with water, located on the inside of the cellular membrane, and are usually membrane-spanning proteins such as channels or pores. Hydrophilic regions are polar and can interact with water, are located on the outside of membranes, and are usually peripheral proteins such as antigens or receptors[8]. The subunit of interest for us is from position 191-209, and as such, from this current plot, it appears that our subunit lies in a hydrophilic region, which makes sense as the location of the R195K variant lies within a transmembrane helical domain. To take a closer look, only the subunit sequence was read into Protscale, both for the normal subunit and the variant version.



The left plot displays the normal subunit and the right plot display the variant subunit, both using the Kyte & Doolittle index and a sliding window of 5. Both plots look virtually the same, which once again makes sense as the point mutation from R to K does not change much in regards to hydropathicity index. According the Kyte & Doolittle index, Arginine has a value of -4.5 while Lysine has a value of -3.9. The R195K variant replaces the most hydrophilic amino acid with the second most hydrophilic amino acid, thus not changing too much in regards to hydropathicity of the subunit or the protein as a whole[9].

Using ExPASy Coils, I predicted the entire CACNA1A protein's likelihood that it would form coils at differing locations. I ran Coils with a window width of all to attempt and find coils using all window sizes, the MTIDK matrix, and no 2.5fold weighting of positions a, d. The following graphic to the right displays that around position 500 there is a highly likely predicted coil for window=21, around position 750 there is a highly likely predicted coil for all windows, and the rest of the predicted coils are for only window=14. However, as our particular subunit of interest lies from position 191-209, these predicted coils do not really play a role in our particular mutation[10].



When using ExPASy Prosite to scan for protein profiles of similar families or domains for

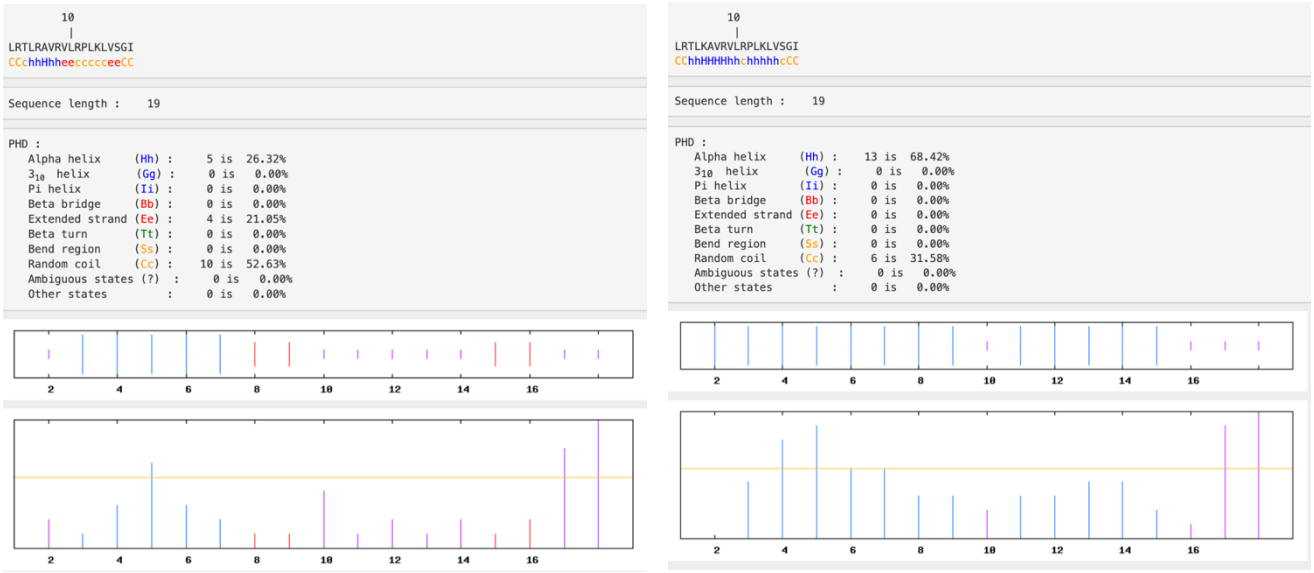
CACNA1A, it returned with no results. Just to double check, I searched using the UniProt accession number for CACNA1A as well as the FASTA file. Both returned with no results[11].

I next wanted to take a closer look at the secondary structure of the CACNA1A gene, the R195K variant, and both the normal S4 and R195K variant S4 subunits. To accomplish this, I first ran both the normal CACNA1A gene FASTA file and the R195K full gene variant FASTA file into PHD. PHD output the following two graphics:

CACNA1A Normal **CACNA1A R195K Variant**

Of important in these two graphics is that the predicted secondary structure did NOT change at all at the location of the R195K variant. Both display high certainty in predicted helical secondary structures as shown by the capital "H's". To get a closer look at the S4 helical subunit, I decided to try and run PHD to predict the secondary structure of just the S4 subunit in which the R195K variant is located, which output the follow two graphics:

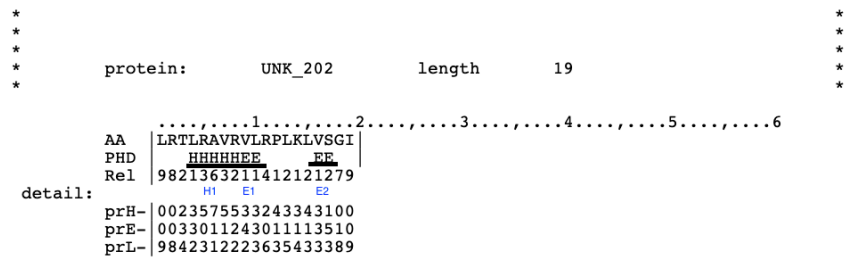
Normal S4 Subunit **R195K Variant S4 Subunit**



Compared to the full gene PHD secondary structure prediction, the secondary structure predictions of just the S4 subunit of the CACNA1A gene shows significant differences. In the normal subunit, PHD predicted that from 191-193, 201-205, and 208-209 are random coils, 194-198 is an alpha helix and 199-200 and 206-207 are extended strands. However, in the R195K variant prediction, PHD predicted a completely different secondary structure. The amount of extended strands, random coils, and alpha helices has changed dramatically. The entire subunit of the protein with the variant R195K has had its predicted secondary structure changed into two portions of alpha helices from 193-201 and 203-207 with random coils in between as opposed to

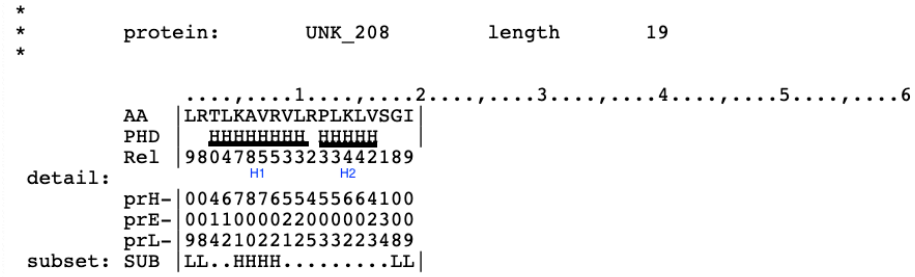
the singular alpha helix subsection with extended strands and random coiling. Additional information from PHD can be seen in the following two graphics for both the Normal S4 subunit and the R195K S4 variant.

Normal S4 Subunit



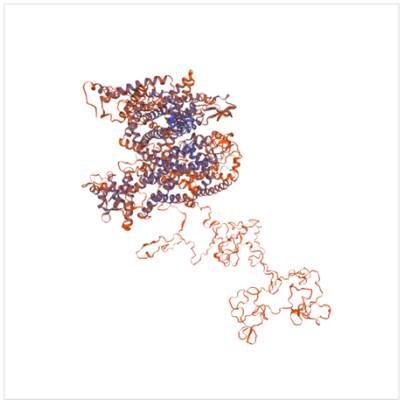
In this graphic from PHD, the certainty of secondary structure prediction can be viewed for each individual residue. It appears that the first helical group H1 has rather low certainty levels (0-9 scale with 9 being the most certain) as most of the residues lie around the 1-3 confidence level with only one of them reaching 6. Similarly, the two extended strands, E1 and E2, both have low certainty levels of prediction. The blank spaces indicate loop conformation predictions which have high certainties on both the front and end of the subunit, but low certainties in the middle, probably a product of the low certainty of prediction for the extended strands that flank it.

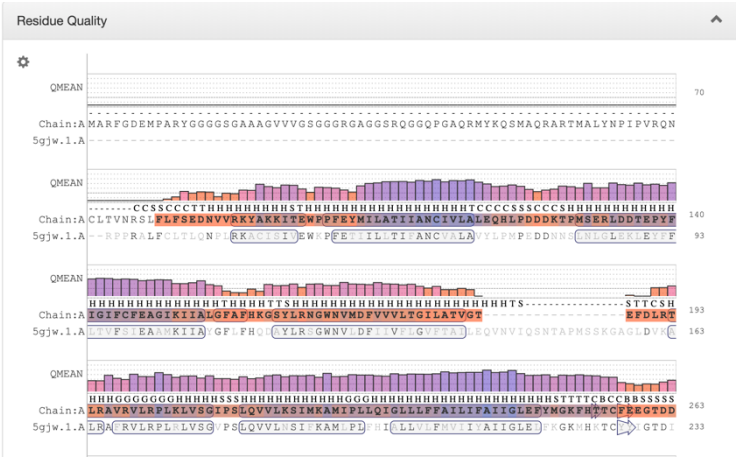
R195K S4 Variant



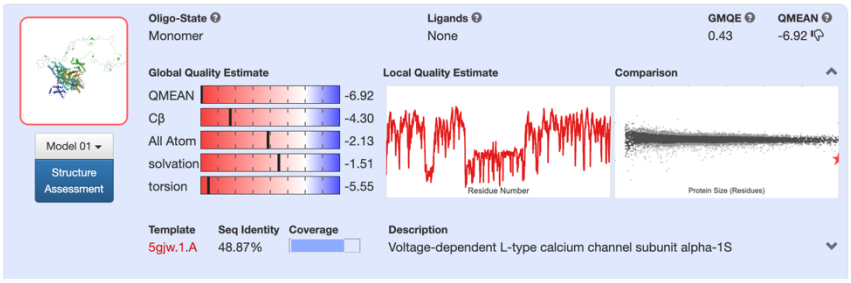
The R195K variant has higher certainty values on average regarding the two helical regions H1 and H2, averaging around 4-5 compared to the secondary structure prediction of the normal subunit 4. The flanking looping regions still maintain their high certainty values. The bottom block displays the highly certain secondary structure predictions with “.’s” displaying areas of low certainty values[12].

The 3D structure of the normal CACNA1A gene was modeled using SWISS-MODEL. In this 3D model, the colors signify the Qmean value for that particular residue. Low Qmean values are displayed in red/orange while high Qmean values are displayed in blue.





As can be seen in the two above graphics, there is a significant portion of orange Qmean scores signifying that the "degree of nativeness" of those residues is low, thus indicating that at that point, the model is of low quality. At residue 195, the location of our R195K variant, the Qmean score is 0.54, which is about in between of a "good" and a "bad" score. The SWISS-MODEL program ran our normal CACNA1A gene sequence against a Voltage-dependent L-type calcium channel subunit alpha-1S and found a sequence identity of 48.87%. The overall global QMEAN of the model is -6.92, indicating that this model is of low quality of prediction. Because the quality of this 3D structure model is so low, and because the R195K variant only switches a single residue out of 2506, I decided to not run the R195K variant through SWISS-MODEL[13].



Discussion

The changes seen in the secondary structure prediction of the normal S4 subunit and the R195K S4 subunit variant may indicate that the predicted change in structure of the protein may be responsible for the dysregulation of calcium ion influx through the Cav2.1 channel. As seen in the PHD secondary structure prediction, the secondary structure of the S4 subunit changes from alternating between coils, a helix, extended strands, and more coils, but the R195K variant's structural prediction displays multiple helices that make up most of the secondary structure. As discussed earlier in this paper, the reason behind familial hemiplegic migraines is a dysregulation of calcium influx which then leads to a dysregulation of neurotransmitter release or uptake. Particularly, the mutations or variants of the Cav2.1 channel cause it to open more easily and not regulate the influx of calcium as well as a normal channel, thus causing the cascade of dysregulation found in hemiplegic migraines. It is possible that the predicted change in secondary structure due to the change of residue at position 195 from R to K leads to a change in function of the Cav2.1 channel in regulation of calcium ions. While Arginine to Lysine does not change drastically in regards to molecular weight or hydropathicity, it is possible that the slightly less hydrophilic Lysine cannot interact quite as well in location 195 and thus causes the structural change seen in the predicted secondary structures. As it has been described that the Cav2.1 channel mutations responsible for hemiplegic migraines is due to the relative ease with which the channel opens and allows calcium ions in, I hypothesize that the secondary structural changes caused by the R195K variant disrupts the proper function of the Cav2.1 channel and either allows calcium ions in more easily or does not regulate CDI properly, thus causing the dysregulation of calcium.

Sources:

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